LHRH AND DOXORUBICIN CONJUGATED GOLD NANOPARTICLES FOR BREAST CANCER TREATMENT

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Background and Objectives: Breast cancer remains the most common cancer among women, accounting for 212,940 new cases and 40,970 deaths in 2006 alone. The primary treatment for cancer is cytoreductive surgery followed by adjuvant chemotherapy, radiotherapy, or both. While this method is successful in the majority of cases, it is accompanied by cytotoxicity to normal cells and organs. The purpose of this study is to develop a method to treat breast cancer more specifically and to minimize the systemic toxicity. Direct targeting of cancer cells can be achieved by using agents specifically directed to binding sites on cancer cells such as carbohydrates, lectins, surface proteins and receptors, etc. LHRH receptors are present in hormone-related tumors including breast cancer. In normal tissues, LHRH receptors are not expressed or expressed at an undetectable level. By coupling these receptors to nanoparticles, breast cancer tumors and their metastases can be targeted directly. We hypothesize that targeting the LHRH receptor using nanoparticles carrying the LHRH analog and cytotoxic agent (doxorubicin) would enhance drug uptake by cancer cells with reduced toxicity to normal cells.

Methods: To test our hypothesis, we conjugated gold nanoparticles (20–30 nm) with LHRH analog "[D-Trp^6]LHRH" and tested for its binding to the LHRH receptor and activation of receptor by performing ligand binding assays and production of intracellular cAMP. The effect of LHRH and doxorubicin conjugated to gold particles on breast tumor cell growth was performed using cell proliferation assays. Distribution of LHRH conjugated particles in tumor, metastatic cells, and other tissues was determined by injection of fluoro-LHRH conjugated gold particles in nude mice bearing s.c. breast tumors followed by examination of tumors and tissues under fluorescence microscope.

Results: Our results showed that conjugation of gold nanoparticles with [D-Trp^6]LHRH and doxorubicin retained its binding affinity for and biological activation of the LHRH receptor. In our studies, we showed that treatment of pituitary gonadotrope tumor cell line LβT2 that express high levels of high affinity LHRH receptors resulted in 87% cell death and loss of cell viability within 48 h at a concentration of 2.5 or 5 nM of conjugated doxorubicin. Similarly, treatment of breast tumor cells "MCF-7" with LHRH and doxorubicin conjugated gold particles showed a significant inhibition of cell growth. Whereas no change in cell proliferation or cell apoptosis was observed in cells treated with equal amounts of [D-Trp^6]LHRH or free doxorubicin. The amount of conjugated doxorubicin required to achieve same level of cell death was found to be 100- to 200-fold lower than the free doxorubicin. Injection of LHRH conjugated fluoro-gold particles (alexa 594 labeled gold particles) into nude mice bearing s.c. tumors and metastatic lung cancer resulted in high accumulation of particles in tumors and metastatic cells in lung. A small amount of particles was observed in pituitary or liver. In contrast unconjugated particles showed high accumulation in liver and very low levels in tumors and lungs.

Conclusions: Results obtained from our studies are very exciting and suggest that [D-Trp^6]LHRH can be specifically used to target breast cancer cells to deliver anticancer agents to induce cell death.

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